FORM PTO-1390 (REV 10-94) U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	9192.5USWO
	U S APPLICATION NO (If known, see 37 C F R 1 5) 09/117071

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB97/00210	January 23, 1997	January 23, 1996

TITLE OF INVENTION
RETROVIRAL VECTOR AND ITS USE IN GENE THERAPY

APPLICANT(S) FOR DO/EO/US

Alan John KINGSMAN and Susan Mary KINGSMAN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(l).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [X] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.

8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [X] An unsigned oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).

10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [X] A FIRST preliminary amendment.
    [ ] A SECOND of SUBSEQUENT preliminary amendment.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. [X] Other items or information: International Preliminary Examination Report; PTO Form 1449; International Search Report and 5 References

U.S. APPLICATION NO. (If known, see 37 C F R 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
	PCT/GB97/00210	9192.5USWO

17. [X] The following fees are submitted:

	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492(a) (1)-(5)):		
Search Report has been prepared by the EPO or JPO **$930.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492(a)(1)) **$720.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$790.00**		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(3)) paid to USPTO **$1,070.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$98.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$930.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	**$**	

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total claims	21	-20 =	1	X **$22.00**	**22.00**	
Independent claims	3	-3 =	0	X **$82.00**	**$0**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ **$270.00**	**$**	
TOTAL OF ABOVE CALCULATIONS =					**$952.00**	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).					**$**	
SUBTOTAL =					**$952.00**	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f).				+	**$**	
TOTAL NATIONAL FEE =					**$952.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property				+	**$**	
TOTAL FEES ENCLOSED =					**$952.00**	
					Amount to be: refunded	**$**
					charged	**$**

a. [X] A check in the amount of $ 952.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-2725. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO
Michael B. Lasky
MERCHANT & GOULD
3100 Norwest Center
90 South Seventh Street
Minneapolis, MN 55403

SIGNATURE

Michael B. Lasky
NAME

29,555
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: KINGSMAN et al. Docket: 9192.5USWO

Filed: Intl Filing Date January 23, 1997

For: RETROVIRAL VECTOR AND ITS USE IN GENE THERAPY

CERTIFICATE UNDER 37 CFR 1.10:
"Express Mail" mailing label number: EL039318174US
Date of Deposit: July 21, 1998

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, D.C. 20231.

By: M. L. Green
Name: Mark Green

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D. C. 20231

Sir:

In connection with the above-identified application filed herewith, please enter the following preliminary amendment:

IN THE ABSTRACT

Insert the attached Abstract page into the application as the last page thereof.

IN THE SPECIFICATION

A courtesy copy of the present specification is enclosed herewith, but the World Intellectual Property Office (WIPO) copy should be relied upon if it is already in the U.S. Patent Office.

IN THE CLAIMS

Please enter the claims as amended in the International Preliminary Examination Report which is attached. Amend the amended claims as follows:

In claim 3, line 1, delete "or claim 2".

In claim 4, line 1, delete "any one of claims 1 to 3" and insert --claim 1--.

In claim 5, line 1, delete "any one of claims 1 to 4" and insert --claim 1--.

In claim 10, line 1, delete "any one of claims 6 to 9" and insert --claim 6--.

In claim 13, line 1, delete "or claim 12".

In claim 14, line 1, delete "or claim 12".

In claim 15, line 1, delete "any one of claims 11 to 13" and insert --claim 11--.

In claim 16, line 1, delete "any one of claims 11 to 14" and insert --claim 11--.

In claim 18, line 3, delete "any one of claims 6 to 10" and insert --claim 6--.

In claim 19, line 3, delete "any one of claims 1 to 5" and insert --claim 1--.

In claim 20, line 1, delete "any one of claims 6 to 10" and insert --claim 6--.

In claim 21, line 2, delete "any one of claims 1 to 5" and insert --claim 1--.

REMARKS

A new abstract page is supplied to conform to that appearing on the publication page of the WIPO application, but the new Abstract is typed on a separate page as required by U.S. practice.

The above preliminary amendment is made to remove multiple dependencies from claims 3- 5, 10, 13-16, and 18 -21.

Applicant respectfully requests that the International Preliminary Examination Report and the preliminary amendment described herein be entered into the record prior to calculation of the filing fee and prior to examination and consideration of the above-identified application.

If a telephone conference would be helpful in resolving any issues concerning this communication, please contact Applicant's primary attorney-of record, Denise M. Kettelberger (Reg. No. 33,924), at (612) 371-5268.

Respectfully submitted,

MERCHANT, GOULD, SMITH, EDELL,
WELTER, & SCHMIDT, P.A.
3100 Norwest Center
90 South Seventh Street
Minneapolis, Minnesota 55402
(612) 332-5300

Dated: July 21, 1998

By ____________________
Michael B. Lasky
Reg. No. 29,555

MBL/sef

Docket No. 9192.5USWO
Intl Filing Date January 23, 1997

Abstract

DNA sequences for creating replication defective retroviral vector producer cells either *in vivo,* or *in vitro* for reimplantation. The retroviral vector comprises at least one heterologous gene and functional *env* and *gag-pol* genes are absent from the vector so that retrovirus structural proteins are not expressed by the target cells infected and transduced by the vector.

RETROVIRAL VECTOR AND ITS USE IN GENE THERAPY

This invention relates to DNA sequences encoding retroviral vectors and to sets of DNA sequences encoding retroviral vector particles. The invention also relates to producer cells containing the sequences and capable of producing the retroviral vector and retroviral vector particles containing the vector and to methods of making the producer cells. The invention further relates to uses of the DNA sequences and producer cells in formulations for gene therapy, and to methods of performing gene therapy using the DNA sequences and producer cells.

INTRODUCTION AND PRIOR ART

A number of diseases are amenable to treatment by the delivery of therapeutic nucleic acids to patient's cells. This is referred to as gene therapy (reviewed extensively in Lever and Goodfellow 1995; Culver 1995; Ledley 1995). To achieve gene therapy there must be a method of delivering genes to the patient's cells and additional methods to ensure the effective production of any therapeutic genes. There are two general approaches to achieve gene delivery; these are non-viral delivery and virus-mediated gene delivery. The best characterised virus-mediated gene delivery system uses replication defective retroviruses to stably introduce genes into patients cells. A major disadvantage of non-viral delivery is that the DNA is confined to the initial target cells and is short lived which, for chronic disease, necessitates repeated treatments with the DNA. A major disadvantage of retroviral vectors is that efficient gene transfer is only achieved by transducing cells *ex vivo* and introducing either the transduced cell population back into the patient or grafting in a cell line that is engineered to release retroviral vector particles. These procedures require significant surgical procedure and manipulation of cells. In addition transduction of patients cells with retroviral vector particles is inefficient.

The various known technologies involved in the field of the invention are described in more detail below.

1. The production of retroviral vectors from multiple separate DNA sequences

It is known that the separate expression of the components of a retroviral vector on separate DNA sequences cointroduced into the same cell will yield retroviral particles carrying defective retroviral genomes that carry therapeutic genes (e.g. Reviewed by Miller 1992). This cell is referred to as the producer cell. There are two common procedures for generating producer cells. In one, the sequences encoding retroviral Gag, Pol and Env proteins are introduced into the cell and stably integrated into the cell genome; a stable cell line is produced which is referred to as the packaging cell line. The retroviral vector genome is then introduced into the packaging cell line by transfection or transduction to create a stable cell line that has all of the DNA sequences required to produce a retroviral vector particle. The second approach is to introduce the three different DNA sequences that are required to produce a retroviral vector particle i.e. the *env* coding sequences, the *gag-pol* coding sequence and the defective retroviral genome into the cell at the same time by transient transfection and the procedure is referred to as transient triple transfection (Landau & Littman 1992; Pear et al 1993;). The triple transfection procedure procedure has been optimised (Soneoka et al 1995; Finer et al 1994). WO 94/29438 describes the production of producer cells *in vitro* using this multiple DNA transient transfection method and describes the use of these producer cells *in vitro* to transfer retroviral particles to human cells of any lineage that have been removed from a patient. WO 94/19478 describes the use of novel cell lines for producing high titre retroviral stocks following the transient transfection of one or more retroviral plasmids into a packaging cell line. This also describes only the transfer of retroviruses to target cells *in vitro*. The transfer of retroviruses from a producer cell to a target cell *in vitro* is referred to as cocultivation and it is a well established procedure for introducing retroviruses into cells *in vitro*.

2. DNA mediated gene delivery *in vivo*.

The delivery of genes into a variety of different cells in man or animals using naked DNA or DNA associated with a non-viral delivery system has been well described (reviewed by Ledley 1995). The simplest method involves injecting naked DNA into tissues where it is taken up by a

proportion of cells and the genes contained in the DNA are expressed to produce proteins in these cells (Dubensky et al 1984; Wolffe et al 1990).

The DNA may be delivered by biolistics; in this procedure metal particles are coated with DNA and projected at high velocity into cells by a high pressure device (e.g. Yang et al 1990). The DNA may be coupled to chemical agents that optimise uptake into cells e.g polylysine or to components of viral particles e.g. adenovirus particles or penton protein or to ligands for specific cognate receptors. The DNA may be encapsulated in liposomes or complexed with cationic lipids (e.g.Hyde et al 1993). Irrespective of how the DNA is delivered by these non-viral methods the seminal feature is that there is no transfer of DNA from the originally transfected cells to other cells except possibly by transfer to daughter cells after cell division. Furthermore the introduced gene is not guaranteed to be permanently maintained in the target cells.

3. Retrovirus mediated gene delivery.

The use of defective retrovirus vectors to deliver genes to target cells is well documented (reviewed by Morgan and Anderson 1993). Defective retroviruses are used to transduce cells that have been removed from the body (*ex vivo* gene delivery) or they can be delivered to tissues *in situ* (*in vivo* gene delivery). The vectors introduce DNA into a cell and it is stably incorporated into the host cell genome where it is expressed to produce any therapeutic gene contained within it. There is no dissemination of the therapeutic gene because retroviral vector mediated gene transfer is a one step event that affects only the initial target cell. *In vivo* gene delivery is not widely used because gene delivery is inefficient largely because the retroviral particles delivered in this way are rapidly cleared from the site of treatment and there is no extended exposure of the cells to viral particles. For example when retroviral vector particles were injected into the brains of rats that were carrying glial tumours only a very few cells were transduced by the vectors due to the short, 2-4hrs, half life of the retroviral particles (Short et al 1990).

4. Implantation of producer cells in target tissues

It has been reported that a producer cell that has been created *in vitro* can be implanted into a tissue *in situ* (Short et al 1990). The producer cell releases retroviral vector particles which then transduce

neighbouring cells. In this procedure a producer cell is created by the stable transformation of the cell with the DNA sequences specifiying retroviral components *in vitro*. The cell is cultured *in vitro* and then surgically implanted in the patient. The producer cell is foreign and may be short lived due to destruction by the immune system.

Clearly there is a need for improved ways and means for introducing therapeutic genes into patients. Gene therapy would be significantly simplified if stable introduction of DNA into patient cells could be achieved following non-viral DNA delivery and if the effectiveness of non-viral DNA delivery could be improved.

WO 96/17053 describes an adenoviral vector capable of tissue-specific replication due to a regulatory sequence operably linked to the coding region of a gene essential for vector replication. The vector can be used to distribute a polynucleotide in a tissue *in vivo*.

WO 95/22617 describes a retrovirus delivery system in which the vector genome contains a therapeutically active gene in place of the *env* gene, but is otherwise identical to the wild type genome. It is suggested that by introducing an *env* gene into the target cell, retrovirus vector particles may then be produced *in situ*.

THE INVENTION

In one aspect, the invention provides a DNA sequence encoding a replication defective retroviral vector for converting cells in a patient into producer cells capable of producing replication defective retroviral vector particles containing the vector, said retroviral vector comprising at least one heterologous gene, which vector does not contain functional *env* or *gag-pol* genes, the DNA sequence in a form suitable for administering to a patient by non-retroviral means and capable of being taken up by the cells.

The invention also provides a set of DNA sequences for converting cells in a patient into producer cells capable of producing replication defective retroviral vector particles, the set of sequences comprising the DNA sequence above and DNA sequences encoding packaging components Env and Gag-Pol for production of infective retroviral vector particles by the producer cells, the set of DNA sequences in a form suitable for administering to a patient by non-retroviral means and capable of being taken up by the cells.

In another aspect, the invention provides a producer cell capable of producing a replication defective retroviral vector in an infective retroviral vector particle, the producer cell comprising a DNA sequence encoding the replication defective retroviral vector, said vector comprising at least one heterologous gene and which vector does not contain functional *env* or *gag-pol* genes, the producer cell being a fresh cell suitable for introduction into a patient and use in gene therapy.

In a further aspect, the invention provides a method of making a producer cell capable of producing a replication defective retroviral vector said vector comprising at least one therapeutically active gene and which vector does not contain functional *env* or *gag-pol* genes, which method comprises introducing a DNA sequence encoding the replication defective retroviral vector into a fresh mammalian cell to give a producer cell suitable for use in gene therapy.

As will be explained below in more detail, conversion of the fresh mammalian cells to producer cells may be carried out either *in vivo* or *in vitro*. In the *in vitro* case, the producer cells will be suitable for implanting into a patient, and are preferably cells from the patient into whom it is intended to reimplant them.

Further aspects of the invention provide methods of performing gene therapy on a patient, comprising introducing into the patient a producer cell, or a DNA sequence or set of sequences, as described herein; and uses of the producer cells and DNA sequences in formulations for use in gene therapy.

It is particularly preferred that the producer cells according to the various aspects of the invention are of a target cell type for which the therapeutically active gene is intended. This avoids the need to introduce any exogenous cells into the target area in a patient.

However, the producer cells may alternatively be cells capable of delivering the therapeutically active gene to the target cell. Cells of the immune system such as macrophages or tumour infiltrating lymphocytes could be converted to producer cells; this would need to be carried out *in vitro*. When reintroduced into the patent these mobile producer cells will infiltrate organs or tissues and retroviral vector particles from the producer cells will infect those organs or tissues.

The term "fresh mammalian cells" as used here refers to mammalian cells which are in their natural state, or as close as possible to

their natural state. Cells which have been extensively cultured *in vitro*, including cell lines, are not considered to be fresh cells. Fresh cells as referred to herein which have been removed from an organism will usually be primary cells in the sense that they are in a primary culture of cells prepared directly from the tissues of an organism and have not been sub-cultured.

It is an important feature of this invention that the replication defective retroviral vector does not encode any of the structural components of retroviral vector particles. The vector thus allows for the insertion of the therapeutically active gene or genes into the target cell genome, without structural genes encoding Env and Gag-Pol. This avoids problems associated with expression of viral components in the target cells, such as undesired immune responses to those components. For example, cytotoxic T cell responses directed against the products of other foreign genes will be avoided.

Thus a set of DNA sequences according to the invention for producing retroviral vector particles having a replication defective retroviral genome, will comprise:

(i) a DNA construct encoding the vector genome, comprising one or more therapeutically active genes plus the remaining components essential for function such as primer binding site, integration sites, packaging signal.

(ii) a DNA construct or constructs encoding Gag-Pol and Env, preferably as separate constructs.

As will be evident to those skilled in the art, safety considerations to avoid generation of replication competent virus through recombination apply and will be taken into consideration in construction of (i) and (ii) above.

The various DNA constructs described above for use in preparing producer cells according to the invention may be present on separate expression vectors, or they may be present on a single expression vector provided that the vector genome is encoded in a separate transcription unit. The expression vector or vectors will usually be plasmids. Preferably, the DNA constructs encoding the retroviral vector and the required packaging components are administered simultaneously to the patient or delivered simultaneously to the cells being converted to

producer cells *in vitro*. It may therefore be more convenient to incorporate all of the elements onto a single expression vector.

The invention thus comprises the combination of two technologies to produce a method of delivering genes directly to patient/animal tissues such that a long term expression of therapeutic products will be achieved. The essence of the invention is that firstly, using non-viral DNA delivery, combinations of DNA sequences are introduced into the patients cells. The DNA sequence encoding the vector itself, that is the genome of the retrovirus particle, lacks functional *env* and *gag-pol* genes so that the vector is safe and practical. The preferred procedure does not require any removal of cells from the patient. The methods described can however be applied to cells or tissues or organs that are removed from the body and then reimplanted. The combination of DNA sequences is such that when expressed they specify the production of a replication defective retroviral vector genome and the production of the protein components that are required to package that genome to produce retroviral vector particles. The retroviral vector particles are released and then by the process of virus-mediated gene delivery they attach to and enter additional cells and consequently deliver the defective retroviral genome into the cell where it is copied by the particle associated reverse transcriptase and becomes integrated into the genome of those cells. The cell that originally receives the combination of DNA molecules continues to secrete retroviral vector particles for as long as the cell survives or the DNA persists. This creates an extended opportunity for the retroviral vector particles to transduce cells. The retroviral vector particles contain a therapeutic gene which is expressed in the transduced cells. The invention therefore involves establishing a retroviral vector producer cell in the target tissue by the direct delivery of appropriate combinations of DNA sequences.

Details

DNA is delivered to the cells by any appropriate non-retroviral method including injection, biolistic delivery, carrier mediated delivery. Some of the known methods have already been described above. A preferred delivery method for delivering DNA sequences to cells *in vivo* is liposome-mediated delivery. Whichever method is used for *in vivo* delivery, the DNA sequences will need to be provided in a

pharmaceutically acceptable formulation for administering to the patient. Multiple different DNA sequences on separate molecules or a single molecule carrying multiple different sequences are delivered to human/animal cells. These DNA sequences encode the components of a retroviral vector for example the HIT system (Soneoka et al 1995) and the kat system (Finer et al 1994). The DNA sequences encode a retroviral Env protein, a retroviral Gag-Pol protein and a replication defective retroviral genome that is engineered to contain one or more therapeutic genes. Additional sequences may also be included. For example a suicide gene such as HSV Tk might be included on one or all molecules to enable transfected and transduced cells to be destroyed by treatment with drugs such as acyclovir (Plautz et al 1991). The combination of DNA sequences are referred to as the vector production system (VPS). The VPS need not be restricted to the three plasmid systems such as HIT and kat but can comprise any retroviral vector system. Components need not be native retroviral components. For example the *env* gene can be engineered to specify an envelope protein that targets retroviral vector particles to a specific cell type, the vector genome can be engineered to contain gene expression signals that confer special properties on the vector e.g. tissue specific expression, regulated expression and the *gag-pol* gene can be engineered to influence infection and integration for example to deliver DNA into the genome of non-dividing cells or to target DNA to a specific site in the chromosome. The cell that receives the VPS is referred to as an *in situ* retroviral factory (ISRF), it is essentially a retroviral vector producer cell created from one of the patient's own cells. If it is created *in vitro*, using cells removed from the patient, the ISRF will need to be provided in a pharmaceutically acceptable formulation for administering to the patient. The ISRF produces retroviral particles that are released from the cell for as long as the VPS persists, this may be of the order of weeks to months or exceptionally years (Wolff et al 1992). The defective retroviral particles transduce neighbouring cells, referred to as the target cell population (TCP), and deliver the therapeutic gene to those cells as a stably integrated provirus. The TCPs do not produce further virus. The ISRF also expresses the therapeutic gene from the VPS. This combination of non-viral DNA delivery and virus-mediated gene delivery as described allows the dissemination of a therapeutic gene safely, throughout a population of patient's cells.

The invention has a number of advantages which relate to the generation of ISRFs in patients cells both in the body and in tissues removed from the body.

Advantages

i) In the patient

1. Increased efficiency of the delivery of retroviral vectors to target cells in the patient because of the local concentration of viral particles.

2. Increased efficiency of the delivery of retroviral vectors because of the extended time period of exposure of cells to viruses. This means that cells at different stages of the cell cycle have the opportunity to cycle into a phase that is optimum for retroviral infection. These cells would not be available as targets in a single dose treatment with retroviral particles.

3. Creation of an ISRF obviates the need to implant a producer cell that has been generated in the laboratory. Such producer cells are different from patients cells and may even be of non-human origin. These cells are rapidly cleared from most sites of implantation in the body and therefore have limited usefulness.

4. The creation of ISRFs dramatically increases the efficiency of non-viral gene therapy methodologies. In these procedures the transient nature of the expression of the therapeutic gene necessitates frequent multiple repeat treatments with DNA. The ISRF will disseminate therapeutic genes to cells that will continue to produce the product for the life-time of the cell. Treatment need therefore be repeated infrequently if at all for some TCPs.

5. Creation of an ISRF obviates the need to surgically remove patient tissues and transduce them with retroviral vectors before reimplantation. This latter procedure does not allow for further dissemination of the therapeutic gene to other cells. It is technically complex and the cells must be subjected to significant manipulation *in vitro* before reimplantation.

6. When applied to the treatment of tumours, the creation of ISRFs increases the probability of obtaining therapeutic gene expression in

the majority of tumour cells and hence increases the probability of tumour clearance.

7. The ISRF technology has a variety of therapeutic uses, for example but not restricted to:-

i) Cystic fibrosis (CF): The VPS is introduced into lung tissue e.g by liposome mediated DNA delivery. The consequently established ISRFs spread the appropriate therapeutic gene e.g. CFTR (cystic fibrosis transmembrane conductance regulator) throughout the pulmonary tissue. This confers extended relief of the pulmonary symptoms of CF.

ii) Parkinsons disease: The VPS is introduced into cells in the brain by biolistic delivery over a small surgically exposed area. The consequently established ISRFs deliver a retroviral vector to specific cells e.g. glial cells or astrocytes to deliver relevant therapeutic genes e.g. Tyrosine hydroxylase and dopa decarboxylase.

iii) Alzheimers disease: The VPS is introduced into cells in the brain. The consequently established ISRFs deliver the appropriate therapeutic gene e.g. Nerve growth factor.

iv) Tumours: The VPS is delivered to the tumour. The consequently established ISRFs deliver a retroviral vector to surrounding tumour cells to deliver relevant therapeutic genes e.g. HSV thymidine kinase (Tk) or foreign histocompatibility antigens.

ii) In patients tissues *ex vivo*

Advantages i) 1-4 also apply to *ex vivo* applications of ISRFs

1. Direct transduction of a patient's cells *ex vivo* with retroviral vector particles requires the large scale production of high titre retroviral vectors and is often not very efficient requiring prolonged cell culture and genetic selection of transduced cells. An alternative approach is the cocultivation of patients cells with a producer cell line that has previously been created *in vitro*. This may necessitate the separation of the target cells from the producer cell before reimplantation of patients cells. The present invention describes a method to convert a patient's cell directly into a producer cell. The retroviral vector particles are then transferred from the producer cell, now referred to as an ISRF, to other patient cells and the organ/tissue/cells can be reimplanted with minimal manipulation. the

creation of ISRFs therefore obviates the need for cocultivation with non-patient cells or treatment of cells with retroviral vector particles in any *ex vivo* method of gene therapy.

The invention will now be further described in the examples which follow.

EXAMPLES

Example 1.

Construction of an ISRF in a Hela cell monolayer

Hela cells are plated into 60cm dishes and allowed to grow to 80% confluence. Plasmid DNA comprising pHIT456, pHIT111 and pHIT60 is prepared for transfer into Hela cells by standard calcium phosphate precipitation and introduced into cells using the extended overlay method as described in detail in Soneoka et al 1995. pHIT456 contains the amphotropic retroviral envelope gene that allows infection of Hela cells, pHIT111 is a defective retroviral genome that contains the *lac*Z gene and pHIT60 contains the MLV *gag-pol* gene. The coexpression of these plasmids results in the production of retroviral vector particles that can transduce target cells with the *lacZ* gene. This is referred to as configuration A. Briefly, 10μg of each plasmid is coprecipitated with calcium phosphate and the resulting precipitate is placed on the Hela cell monolayer. After 24 hrs the medium is removed and replaced with fresh medium. Replicate dishes are taken at 24hr intervals and the cells are fixed and stained with X-gal to detect the expression of β-galactosidase (Sanes et al 1986). In a control experiment 10μg of plasmid pKV469 is used in place of the retroviral vector plasmid pHIT111. pKV469 is a simple eukaryotic cell expression vector that expresses the *lac*Z gene via the CMV-IE promoter. In this three plasmid configuration no retroviral vector particles are produced. This is referred to as configuration B.

Cells that are expressing β-galactosidase are stained blue with X-gal. After 24hrs β-galactosidase is expressed in both configurations from the vector plasmid pHIT111 and from pKV469. When the cells are

counted a similar number is observed in each configuration. After 48hrs there is an increase in the number of blue cells in both cases. In configuration B this is due to cell division and adjacent pairs (doublets) of blue cells are observed. In configuration A there is also an increase in the number of cells but this comprises both an increase in doublet cells and an increase in single cells and also the appearance of foci of blue cells. The foci comprise more than two cells which could not result from gene transfer by cell division. The foci appear because virus released from the original cells has infected neighbouring cells. The increase in blue cells in configuration A is more marked after 48 hours with multicellular foci and increased numbers of single cells appearing. This pattern of staining is indicative of one or more rounds of retroviral transduction occurring after the initial transfection of the DNA into the Hela cells. In configuration A, ISRFs are established in the Hela cell monolayer and the *lac*Z gene is disseminated through the target cell population. In configuration B, β-galactosidase expression is resticted to the initially transfected cells and some of their progeny. This experiment establishes that repeated retroviral transduction can occur in a simple homogeneous cell population without the addition of fresh cells as would be the case in a standard cocultivation experiment.

Example 2

Dissemination of the *lac*Z gene throughout the pulmonary tissues of mice

The configuration A and configuration B plasmid sets as above are complexed with cationic liposomes DOTAP or DOTMA/DOPE as described by Alton et al 1993 using 10 to 50μg per plasmid. Liposomes containing DNA are introduced into the lungs of the Edinburgh CF transgenic mouse (Dorin et al 1992) using a jet nebuliser (Alton et al 1993). Mice are sacrificed after 2 days and epithelial cells are harvested by pulmonary lavage. This is repeated for replicate mice at 4 and 14 days. At 14 days lungs are sectioned and sections are stained for the presence of β-galactosidase in pulmonary tissue. In configuaration A the number of blue cells increases to a significantly greater extent than in configuration B

and in histological sections foci of blue cells are seen in configuration A but not in configuration B. An ISRF has been established with configuration A and the *lac*Z gene is disseminated through lung tissue.

Example 3

Dissemination of the *lac*Z gene throughout the liver of mice

Mice are subjected to partial hepatectomy. Plasmids in configurations A and B are precipitated by calcium phosphate in the presence of 1μm gold particles. Gold particles are delivered to cells using a biolisitic delivery device. After 2, 4 and 14 days animals are sacrificed and liver sections are stained with X-gal. Foci and scattered blue cells are seen in the liver in configuration A only.

Example 4

Dissemination of the *lac*Z gene throughout the colon of mice

Plasmids in configuration A and B are complexed with cationic liposomes and these are delivered to the colon by instillation. After 2, 4 and 6 days animals are sacrificed and histological sections of the colon are stained with X-gal. Foci and scattered blue cells are seen in colonic epithelium.

Example 5

Dissemination of the *lac*Z gene into the brains of mice.

Plasmids in configuration A and B are introduced in the brains of mice through a surgical window in the cranium. DNA is delivered by a biolistic device. Mice are sacrificed after 4 days and 4 weeks. Foci and scattered blue cells are seen with configuration A only.

Example 6

ISRF using human HT1080 cells in culture:

Three-plasmid co-transfections were carried out by calcium-phosphate precipitation as described in Soneoka *et al.* (1995). Plasmid pHIT 60 (MuLV *gag-pol* expression plasmid), pHIT 456 (amphotropic *env* expression plasmid), and pHIT 111 (proviral DNA construct containing the *lacZ* gene) were co-transfected into HT1080 cells in duplicate sets of five 10cm dishes. In the first instance, these cells were maintained for five days. Every day, one dish from one set was fixed and stained with X-gal, and one dish from the other set was harvested and lysed to measure β-galactosidase activity by a colorimetric assay. As a negative control, HT1080 cells were transfected with pHIT 60, pHIT 123 (ecotropic *env* expression plasmid), and pHIT 111, and the same assays were performed as for the amphotropic producers as described above. Another set of five 10cm dishes was mock-transfected and one dish was harvested everyday to monitor cell growth by counting the number of cells using a hemacytometer. The results obtained are presented in Table 1. The amphotropic virus-producing HT1080 cells showed an increase in *lacZ*-expressing cells and β-galactosidase activity after one day and the levels were maintained thereafter up to day 5. The increase in both *lacZ*-expressing cells and β-galactosidase activity could not be attributed solely to the increase in cell number, since there was no significant cell growth after day 2 and since the ecotropic virus-producing HT1080 cells showed a reduction in the number of *lacZ*-expressing cells (Table 1). In these experiments, polybrene was not used to enhance virus transduction and titers obtained without the use of polybrene at the standard time of 48 hours post-transfection (day 1) were relatively low, approximately 10^3 LFU/ml on NIH 3T3 cells for both ecotropic and amphotropic viruses, and also on HT1080 cells with the amphotropic virus. Therefore, it can be concluded that in one 10cm dish containing 5ml of media, up to 5×10^3 transducible particles were in suspension capable of spreading to surrounding HT1080 cells.

The procedure was repeated but the cells were maintained for 14 days. The transfected cells were passaged on days 5 and 10 at a ratio of 1:5. Again, a similar phenomenon was observed with amphotropic virus-producing HT1080 cells, in that β-galactosidase activity of cells increased after day 1 and was maintained thereafter (Table 2). A significant increase in activity was observed at day 12 (Table 2). This

increase was most likely due to proliferation of cells harboring the *lacZ* gene, rather than the spread of retroviral vectors, as no infectious virus was being produced at this stage (Table 2). β-galactosidase activity of ecotropic virus-producing HT1080 cells remained at almost baseline level throughout the 14 days, suggesting that the *lacZ* gene transduced by the amphotropic virus was maintained and stably expressed.

No significant cell growth appeared to occur during the course of either experiment, except only after the first day and probably after passage of the cells on days 5 and 10 in the second experiment (Tables 1 and 2). Cells were transfected at approximately 80% confluency, since some toxicity appears to occur from the calcium-phosphate transfection. Recovery of cells from the transfection may explain the growth of cells between days 1 and 2. By day 2, the cells were nearing confluency, hence, the lack of detectable cell growth. Although the nature of MuLV requires that cells be in an actively-dividing state for infection to occur, the low virus titers produced from day 3 onwards (Table 2) suggests that growth of the cells after day 2 was insignificant.

Taken together these data demonstrate that it is possible to use in situ retroviral factories as effective means of spreading useful retroviral vectors through a population of cells.

Table 1 Spread of retroviral vector in HT1080 retroviral producer cells maintained for 5 days

TYPE OF VIRUS PRODUCED[a]	DAY[b]	# OF BLUE CELLS[c]	β-GAL ACTIVITY[d]	# OF CELLS[e]
Amphotropic	1	4	0.02	N/A[f]
(pHIT60,	2	10.4	0.25	N/A
pHIT456,	3	10.4	0.22	N/A
pHIT111)	4	11.2	0.22	N/A
	5	10.1	0.24	N/A
Ecotropic	1	4.5	0.02	N/A
(pHIT60,	2	1.6	0	N/A
pHIT123	3	1	0	N/A
pHIT111)	4	0.7	0	N/A
	5	0.6	0	N/A
Mock transfected	1	N/A	N/A	3.4×10^6
	2	N/A	N/A	7.3×10^6
	3	N/A	N/A	4.0×10^6
	4	N/A	N/A	5.7×10^6
	5	N/A	N/A	7.0×10^6

[a] Three-plasmid co-transfections were performed as described.
[b] Day 1 corresponds to 48 hours post-transfection.
[c] Cells were fixed and stained with X-gal. The number of blue cells was counted under x40 magnification and the average of 10 fields was recorded.
[d] 100μg of total protein extract was used and absorbance was measured at OD_{420}.
[e] Total number of cells in a 10cm dish was determined using a hemacytometer.
[f] Not applicable.

Table 2 Spread of retroviral vector in HT1080 retroviral producer cells maintained for 14 days

VIRUS TYPE[a]	DAY[b]	β-GAL ACTIVITY[c]	TITERS[d] NIH 3T3	# OF HT1080	CELLS[e]
Amphotropic	1	0.023	3.4×10^3	3×10^2	N/A[f]
	2	0.036	ND[g]	ND	N/A
	3	0.094	3.8×10^2	53	N/A
	4	0.061	ND	ND	N/A
	5	ND	1	0	N/A
	8	0.053	0	0	N/A
	12	0.355	0	0	N/A
	14	0.310	0	0	N/A
Ecotropic	1	0.014	1.8×10^3	0	N/A
	2	0.033	ND	ND	N/A
	3	0.018	3.8×10^2	0	N/A
	4	0.026	ND	ND	N/A
	5	ND	0	0	N/A
	8	0.008	0	0	N/A
	12	0.014	0	0	N/A
	14	0.074	0	0	N/A
Mock-transfected	1	N/A	N/A		2.7×10^6
	2	N/A	N/A		7.2×10^6
	3	N/A	N/A		7.5×10^6
	4	N/A	N/A		7.4×10^6
	8	N/A	N/A		8.7×10^6
	12	N/A	N/A		4.4×10^6
	14	N/A	N/A		6.2×10^6

[a] Three-plasmid co-transfections were performed as described . Amphotropic: pHIT60, pHIT456, pHIT111; Ecotropic: pHIT60, pHIT123, pHIT111.

[b] Day 1 corresponds to 48 hours post-transfection. Cells were split at a ratio of 1:5 on days 5 and 10.

[c] 30μg of total protein extract was used and absorbance was measured at OD_{420}.

[d] Titers were obtained by harvesting supernatant from each 10cm dish, filtering through 0.45μm filters, and adding viral supernatant to either NIH 3T3 cells or HT1080 cells in the absence of polybrene. Cells were X-gal stained 48 hours later and titers were obtained in *lacZ*-forming units per ml (LFU/ml).

[e] Total number of cells from mock-transfected 10cm dishes was counted using a hemacytometer.

[f] Not applicable.

[g] Not done.

REFERENCES

Alton, E. et al 1993 Nature Genet. 5, 135
Dorin, J.R. et al 1992. Trans. Res. 1, 101.
Dubensky, T.W. 1984. Proc. Natl. Acad. Sci. 81, 7529
Finer M. et al. 1994. Blood, 83, 43.
Hyde, S.C 1993, Nature 362, 250
Landau, N.R. & Littman, D. 1992. J. Virol. 66, 5110
Ledley, F.D. 1995. Hum. Gene Ther. 6, 1129.
Lever and Goodfellow 1995; Br. Med Bull., 51, 1-242;
Miller, A.D. 1992. Curr. Topics in Microbiol and Immunol. 158, 1-24
Morgan and Anderson. 1993. Annual Review of Biochem., 62, 192
Pear W.S. et al 1993. Proc. Natl. Acad. Sci. 90, 8392
Plautz G. et al. 1991 New Biol.7, 709
Sanes, J.R., et al . 1986 EMBO J., 5 3133.
Short M.P.et al 1990. J. Neuroscience Res. 27, 427
Soneoka Y. et al. 1995. Nucl. Acids Res. 23, 628
Wolff, J.A. 1990. Science, 247, 1465.
Yang, N.S. et al 1990, Proc. Natl. Acad. Sci, 87, 9568.

CLAIMS:

1. A DNA sequence encoding a replication defective retroviral vector for converting cells in a patient into producer cells capable of producing replication defective retroviral vector particles containing the vector, said retroviral vector comprising at least one heterologous gene, which vector contains neither functional *env* nor functional *gag-pol* genes, the DNA sequence in a form suitable for administering to a patient by non-retroviral means and capable of being taken up by the cells, said DNA sequence for use in treatment.

2. A set of DNA sequences for converting cells in a patient into producer cells capable of producing replication defective retroviral vector particles, the set of sequences comprising the DNA sequence according to claim 1 and DNA sequences encoding packaging components Env and Gag-Pol for production of infective retroviral vector particles by the producer cells, the set of DNA sequences in a form suitable for administering to a patient by non-retroviral means and capable of being taken up by the cells, said set of DNA sequences for use in treatment.

3. DNA sequences according to claim 1 or claim 2, wherein the at least one heterologous gene includes at least one therapeutically active gene.

4. DNA sequences according to any one of claims 1 to 3, for converting cells of the patient which are of a target cell type intended for receiving the therapeutically active gene.

5. DNA sequences according to any one of claims 1 to 4, present in one or more plasmids.

6. A producer cell for use in treatment, said producer cell capable of producing a replication defective retroviral vector in an infective

retroviral vector particle, the producer cell comprising a set of DNA sequences encoding the replication defective retroviral vector and the packaging components Env and Gag-Pol, said vector comprising at least one heterologous gene and which vector contains neither function Env nor functional *gag-pol* genes, the producer cell being a fresh cell suitable for introduction into a patient and use in gene therapy.

7. The producer cell according to claim 6, wherein the at least one heterologous gene in the vector includes at least one therapeutically active gene.

8. The producer cell according to claim 7, wherein the cell is of a target cell type intended for receiving the therapeutically active gene.

9. The producer cell according to claim 7, wherein the cell is an immune system cell capable of delivering the vector to target cells intended to receive the therapeutically active gene.

10. The producer cell according to any one of claims 6 to 9, for reimplantation into the patient from which it is derived.

11. An *in vitro* method of making a producer cell capable of producing a replication defective retroviral vector in an infective retroviral particle, said vector comprising at least one therapeutically active gene and which vector contains neither functional *env* nor functional *gag-pol* genes, which method comprises introducing a set of DNA sequences encoding the replication defective retroviral vector and packaging components Env and Gag-Pol into a fresh mammalian cell *in vitro* to give a producer cell suitable for use in gene therapy.

12. The method according to claim 11, wherein the producer cell is of a target cell type intended for receiving the therapeutically active gene.

13. The method according to claim 11, wherein the producer cell is an immune system cell capable of delivering the vector to target cells intended to receive the therapeutically active gene.

14. The method according to any one of claims 11 to 13, wherein the fresh cell is from a patient to be treated by gene therapy.

15. Use of a producer cell according to any one of claims 6 to 10, in the manufacture of a medicament for use in gene therapy.

16. Use of a DNA sequence or set of DNA sequences according to any one of claims 1 to 5, in the manufacture of a medicament for use in gene therapy.

17. A method of making a producer cell *in vivo* in a patient, which producer cell is capable of producing a replication defective retroviral vector in an infective retroviral particle, said vector comprising at least one therapeutically active gene and which vector contains neither functional *env* nor functional *gag-pol* genes, which method comprises introducing a set of DNA sequences encoding the replication defective retroviral vector and packaging components Env and Gag-Pol into at least one cell of the patient *in vivo* to give a producer cell.

18. The method according to claim 17, wherein the producer cell is of a target cell type intended for receiving the therapeutically active gene.

19 The method according to claim 17, wherein the producer cell is an immune system cell capable of delivering the vector to target cells intended to receive the therapeutically active gene.

20. A method of performing gene therapy on a patient, which method comprises introducing into the patient a producer cell according to any one of claims 6 to 10.

21. A method of performing gene therapy on a patient, which method comprises introducing into the patient by non-retroviral means a set of DNA sequences according to any one of claims 2 to 5.

Attorney Docket No. 9192.5USWO

MERCHANT, GOULD, SMITH, EDELL, WELTER & SCHMIDT

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: RETROVIRAL VECTOR AND ITS USE IN GENE THERAPY

The specification of which
a. ☐ is attached hereto
b. ☒ was filed on as application serial no. and was amended on (if applicable) (in the case of a PCT-filed application) described and claimed in international no. PCT/GB97/00210 filed Intl Filing Date January 23, 1997 and as amended on (if any), which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (attached hereto).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

a. ☐ no such applications have been filed.
b. ☒ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119			
COUNTRY	**APPLICATION NUMBER**	**DATE OF FILING (day, month, year)**	**DATE OF ISSUE (day, month, year)**
Great Britain	9601336.2	January 23, 1996	
Great Britain	9620759.2	October 4, 1996	
ALL FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	**APPLICATION NUMBER**	**DATE OF FILING (day, month, year)**	**DATE OF ISSUE (day, month, year)**

I hereby claim the benefit under Title 35, United States Code, § 120/365 of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

U.S. PROVISIONAL APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)

I hereby appoint the following attorney(s) and/or patent agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

Albrecht, John W.	Reg. No. 40,481	Lacy, Paul E.	Reg. No. 38,946
Ansems, Gregory M.	Reg. No. P-42,264	Larson, James A.	Reg. No. 40,443
Batzli, Brian H.	Reg. No. 32,960	Lasky, Michael B.	Reg. No. 29,555
Beard, John L.	Reg. No. 27,612	Lindquist, Timothy A.	Reg. No. 40,701
Berman, Charles	Reg. No. 29,249	Lynch, David W.	Reg. No. 36,204
Black, Bruce E.	Reg. No. P-41,622	McDaniel, Karen D.	Reg. No. 37,674
Blasdell, Thomas L.	Reg. No. 31,329	McDonald, Daniel W.	Reg. No. 32,044
Bogucki, Raymond A.	Reg. No. 17,426	McIntyre, Iain A.	Reg. No. 40,337
Bruess, Steven C.	Reg. No. 34,130	Mueller, Douglas P.	Reg. No. 30,300
Byrne, Linda M.	Reg. No. 32,404	Nasiedlak, Tyler L.	Reg. No. 40,099
Canady, Karen S.	Reg. No. 39,927	Nelson, Albin J.	Reg. No. 28,650
Carlson, Alan G.	Reg. No. 25,959	Orler, Anthony J.	Reg. No. 41,232
Carter, Charles G.	Reg. No. 35,093	Pauly, Daniel M.	Reg. No. 40,123
Caspers, Philip P.	Reg. No. 33,227	Plunkett, Theodore	Reg. No. 37,209
Chiapetta, James R.	Reg. No. 39,634	Reich, John C.	Reg. No. 37,703
Clifford, John A.	Reg. No. 30,247	Reiland, Earl D.	Reg. No. 25,767
Daignault, Ronald A.	Reg. No. 25,968	Rittmaster, Ted R.	Reg. No. 32,933
Daley, Dennis R.	Reg. No. 34,994	Schmaltz, David G.	Reg. No. 39,828
Dalglish, Leslie E.	Reg. No. 40,579	Schuman, Mark D.	Reg. No. 31,197
Daulton, Julie R.	Reg. No. 36,414	Schumann, Michael D.	Reg. No. 30,422
DeVries Smith, Kate	Reg. No. P-42,157	Sebald, Gregory A.	Reg. No. 33,280
DiPietro, Mark J.	Reg. No. 28,707	Sharp, Janice A.	Reg. No. 34,051
Edell, Robert T.	Reg. No. 20,187	Skoog, Mark T.	Reg. No. 40,178
Epp Ryan, Sandra	Reg. No. 39,667	Smith, Jerome R.	Reg. No. 35,684
Farber, Michael B.	Reg. No. 32,612	Soderberg, Richard	Reg. No. P-43,352
Funk, Steven R.	Reg. No. 37,830	Sumner, John P.	Reg. No. 29,114
Glance, Robert J.	Reg. No. 40,620	Sumners, John S.	Reg. No. 24,216
Golla, Charles E.	Reg. No. 26,896	Tellekson, David K.	Reg. No. 32,314
Gorman, Alan G.	Reg. No. 38,472	Trembath, Jon R.	Reg. No. 38,344
Gould, John D.	Reg. No. 18,223	Underhill, Albert L.	Reg. No. 27,403
Gregson, Richard	Reg. No. P-41,804	Vandenburgh, J. Derek	Reg. No. 32,179
Gresens, John J.	Reg. No. 33,112	Victor, David W.	Reg. No. 39,867
Hamre, Curtis B.	Reg. No. 29,165	Welter, Paul A.	Reg. No. 20,890
Hillson, Randall A.	Reg. No. 31,838	Whipps, Brian	Reg. No. P-43,261
Johnston, Scott W.	Reg. No. 39,721	Williams, Douglas J.	Reg. No. 27,054
Kastelic, Joseph M.	Reg. No. 37,160	Witt McDonald, Jonelle	Reg. No. P-41,980
Kettelberger, Denise	Reg. No. 33,924	Wood, Gregory B.	Reg. No. 28,133
Komanduri, Janaki	Reg. No. 40,684	Wood, William J.	Reg. No. P-42,236
Kowalchyk, Alan W.	Reg. No. 31,535	Xu, Min S.	Reg. No. 39,536
Kowalchyk, Katherine M.	Reg. No. 36,848		

I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Merchant, Gould, Smith, Edell, Welter & Schmidt to the contrary.

Please direct all correspondence in this case to Merchant, Gould, Smith, Edell, Welter & Schmidt at the address indicated below:

Merchant, Gould, Smith, Edell,
Welter & Schmidt
3100 Norwest Center
90 South Seventh Street
Minneapolis, MN 55402-4131

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2	**Full Name Of Inventor**	**Family Name** Kingsman	**First Given Name** Alan	**Second Given Name** John
0	**Residence & Citizenship**	**City** Oxon	**State or Foreign Country** United Kingdom GB	**Country of Citizenship** Great Britain
1	**Post Office Address**	**Post Office Address** Greatystones, Middle Street, Islip	**City** Oxon	**State & Zip Code/Country** OX5 2SF United Kingdom
Signature of Inventor 201:			**Date:** 24/7/98	

2	**Full Name Of Inventor**	**Family Name** Kingsman	**First Given Name** Susan	**Second Given Name** Mary
0	**Residence & Citizenship**	**City** Oxon	**State or Foreign Country** United Kingdom GB	**Country of Citizenship** Great Britain
2	**Post Office Address**	**Post Office Address** Greystones, Middle Street, Islip	**City** Oxon	**State & Zip Code/Country** OX5 2SF United Kingdom
Signature of Inventor 202:			**Date:** 29/7/98	

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)–(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) It refutes, or is inconsistent with, a position the applicant takes in:

(i) Opposing an argument of unpatentability relied on by the Office, or

(ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden–of–proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) Each inventor named in the application:

(2) Each attorney or agent who prepares or prosecutes the application; and

(3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.